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A New Species of *Pedibothrium* (Cestoidea: Tetraphyllidea) from the Short-tail Nurse Shark, *Pseudoginglymostoma brevicaudatum* (Elasmobranchii: Orectilobiformes), from Southwest Madagascar

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ABSTRACT: Examination of the contents of the spiral intestine of a preserved specimen of the short-tail nurse shark, *Pseudoginglymostoma brevicaudatum,* collected in the Mozambique Channel off Toliara, Madagascar, led to the discovery of *Pedibothrium toliarensis* sp. n. Among the 9 described species of *Pedibothrium,* the new species most closely resembles *Pedibothrium longispine* in its possession of bipronged hooks with axial and abaxial prongs that are conspicuously recurved. It is readily distinguished from the latter species in its possession of a greater number of testes, a larger scolex, and 2 (rather than 1) columns of testes in the postporal region of the segment. Species of *Pedibothrium* are now known from 3 of the 5 species of sharks belonging to the orectilobiform family Rhincodontidae. This record expands the known geographic range of the host species and parasite genus to include the southwestern coast of Madagascar. Several strobilar fragments consistent with the segment morphology of *Pedibothrium,* but differing from *P. toliarensis* in genital pore position and testes number, suggest that *P. brevicaudatum* may host additional species of *Pedibothrium.*

KEY WORDS: *Pedibothrium,* Tetraphyllidea, Onchobothriidae, *Pseudoginglymostoma,* Madagascar.

To date, the genus *Pedibothrium* includes 9 species of tapeworms. Verified host records for these 9 species are restricted to sharks belonging to the orectilobiform family Rhincodontidae. This family of sharks is interesting in that it currently consists solely of 5 monotypic genera. According to the most recent cladistic treatment of the group (see Dingerkus, 1986), these 5 shark species represent 3 lineages: (1) the Atlantic nurse shark (*Ginglymostoma cirratum* (Bonnaterre, 1788)) plus the tawny nurse shark (*Nebrius ferrugineus* (Lesson, 1830)), (2) the whale shark (*Rhincodon typus* Smith, 1828) plus the zebra shark (*Stegostoma fasciatum* (Hermann, 1783)), and (3) a basal lineage consisting solely of the short-tail nurse shark (*Pseudoginglymostoma brevicaudatum* (Günther, 1866)). The 9 known species of *Pedibothrium* have been reported from 2 of the 5 shark species in the family representing each of the 2 nonbasal lineages of rhincodontids. According to Caira (1992), *G. cirratum* hosts: *Pedibothrium manteri* Caira, 1992; *Pedibothrium brevispine* Linton, 1909; *Pedibothrium longispine* Linton, 1909; *Pedibothrium globicephalum* Linton, 1909; *Pedibothrium maccallumi* Caira and Pritchard, 1986; and *Pedibothrium servattorum* Caira, 1992. *Stegostoma fasciatum* hosts *Pedibothrium veravalensis* Shinde, Jadhav & Deshmukh, 1980; *Pedibothrium lintoni* Shinde, Jadhav & Deshmukh, 1980 (see Shinde et al., 1980; Butler, 1987; and Caira, 1992); and possibly also *Pedibothrium kerkhami* (see Caira, 1992). These records suggest that *Pedibothrium* is restricted to sharks of the family Rhincodontidae, but the extent of the association with sharks of this host family is unclear because, to date, no onchobothriid records exist for *N. ferrugineus, R. typus,* or *P. brevicaudatum.* In this study, we focused on the parasites of *P. brevicaudatum* in particular because of the basal position of this shark taxon on the rhincodontid tree. As we accept the proposal of Baer and Euzet (1962) that *Pedibothrium hutsoni* Southwell, 1911 belongs in the genus *Pachybothrium* (see also Caira and Pritchard, 1986), we have omitted this species from consideration.

According to Compagno (1984), *P. brevicaudatum* is known only from the east coast of Tanzania and Kenya, and possibly the Mauritius and Seychelle islands. However, a collecting trip to Madagascar conducted in 1997 revealed not only that *P. brevicaudatum* is present in the waters of that country, but also that this shark species hosts at least 1 new species of *Pedibothrium* described below.

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Materials and Methods

Interviews of fishermen working in the vicinity of Toliara, Madagascar, suggested that *P. brevicaudatum* (locally known as "Hia Hia") is a fairly common species in that region. We were unable to obtain freshly killed specimens of *P. brevicaudatum.* However, Dr. Edward Mare, the director of the museum at the Institut Halieutique et des Sciences Marines de l'Université de Toliara, kindly allowed us to necropsy a preserved museum specimen of *P. brevicaudatum.* This specimen had been caught off the coast of Toliara and had been stored in ethanol following fixation in formalin. The body cavity and spiral intestine had been opened with longitudinal incisions prior to fixation and thus fixative had penetrated into the internal regions of the spiral intestine when the shark was placed in formalin. To minimize disturbance of the host specimen, the spiral intestine was left attached within the body of the shark. Contents of the spiral intestine were removed through the existing longitudinal incision with a small currette and examined with a dissecting microscope. Worms collected from the contents of the spiral intestine were transferred to 70% ethanol for storage. Specimens prepared as whole mounts for light microscopy were hydrated in a graded ethanol series, stained in Gill's hematoxylin, dehydrated in a graded ethanol series, cleared in xylene, and mounted in Canada balsam on glass slides. The scolex of 1 specimen was prepared for scanning electron microscopy according to the procedure described by Freidenfelds et al. (1994). The dried specimen was attached to an aluminum stub with carbon tape, further grounded with carbon paint, sputter coated with approximately 200 Å of gold/palladium, and examined with a LEO/Zeiss DSM982 Gemini field emission scanning electron microscope. The stub was retained in the personal collection of the senior author.

All measurements are in micrometers unless otherwise stated and are given in the text as the range followed in parentheses by the mean, the standard deviation, the number of worms examined, and the number of observations made if more than 1 measurement for any character was taken per individual worm. Hook measurements were made following Caira (1992). Illustrations were prepared with the aid of a drawing tube. Abbreviations used for museums are as follows: DAB, Department of Animal Biology, % Dr. Daniel Rakotondravony, University of Antananarivo, Antananarivo, Madagascar; MNHN, Collection du Museum National d'Historie Naturelle, Paris, France; USNPC, United States National Parasite Collection, Beltsville, Maryland, U.S.A.

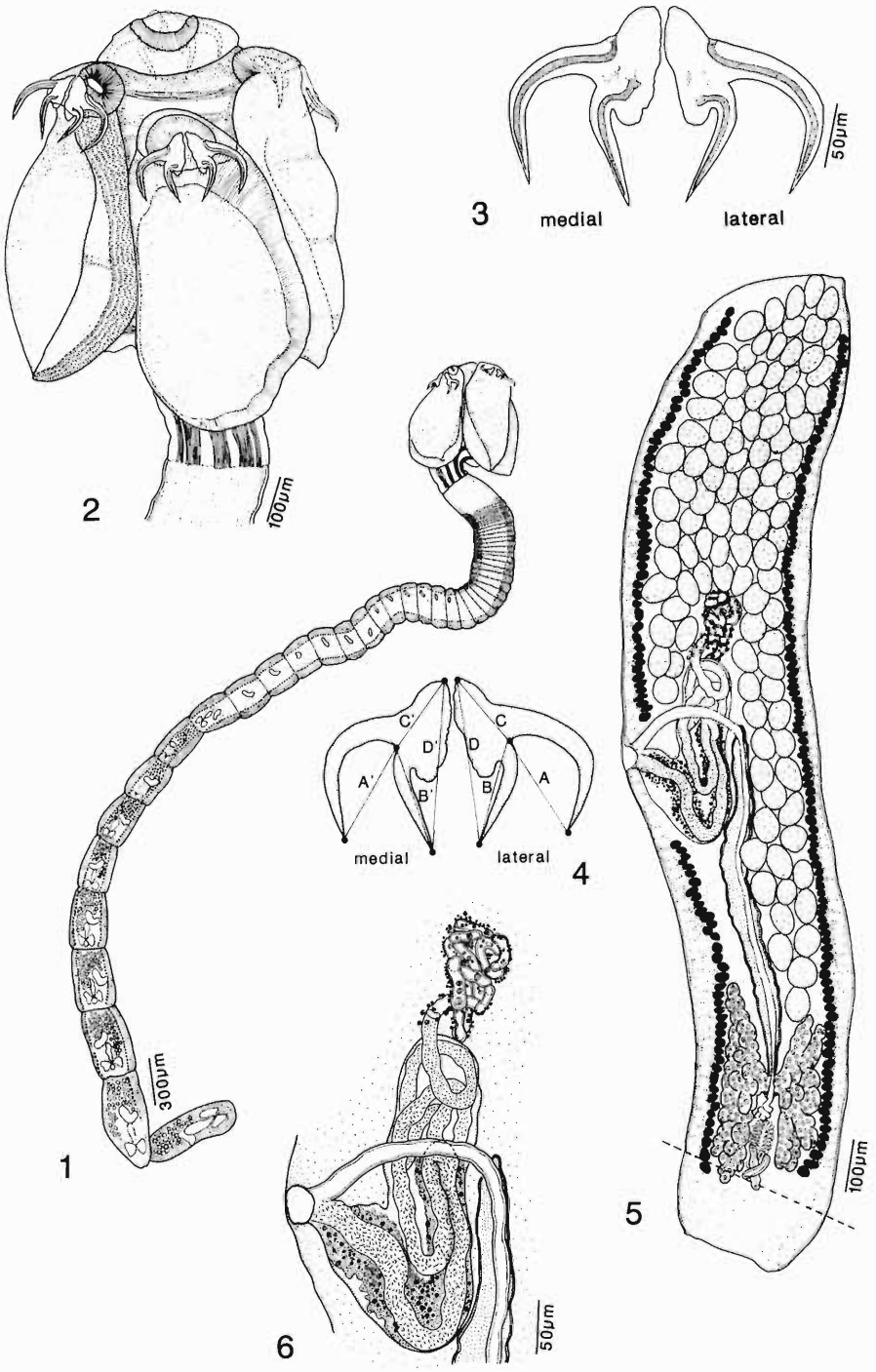
Results

Six tapeworm specimens with scoleces, 6 strobilar fragments, and 3 free segments were found among the debris removed from the spiral intestine of the specimen of *P. brevicaudatum.* The 6 specimens with scoleces, 1 of the strobilar fragments, and 1 of the free segments were considered to represent the new species of *Pedibothrium* described below.

Pedibothrium toliarensis sp. n. (Figs. 1–6)

DESCRIPTION: (based on 1 complete specimen, 4 incomplete specimens with scoleces, 1 strobilar fragment, 1 free segment, and 1 scolex examined with SEM): Worms 1,950 (n = 1) long; greatest width at level of scolex (Fig. 1); 23–51 (39 ± 14.6; 3) segments per worm; acraspedote, euapolytic; genital pores marginal, 42–44% (43 ± 0.8; 2; 2) of segment length from posterior end, irregularly alternating. Scolex with 2 dorsal and 2 ventral bothridia supported on conspicuous cephalic peduncle (Fig. 2). Each bothridium elongate oval in outline with rounded posterior margins, 698–833 (750 ± 33.1; 5; 18) long by 335–434 (390 ± 31.1; 5; 8) wide; consisting of 1 posthook loculus and anterior muscular pad with apical sucker (Fig. 2). Muscular pad 72 (n = 1) long by 185 (n = 1) wide; accessory sucker 39–48 (43 ± 5.2; 2; 3) long by 100–146 (116 ± 20.6; 2; 4) wide; loculus 536–704 (637 ± 45.9; 5; 17) long. Lateral margins of loculi well defined, not folded over one another along axis of bothridium. One pair of symmetrical bipronged hooks at boundary between muscular pad and loculus of each bothridium. Lateral and medial bothridial hooks similar in shape. Abaxial and axial prongs of each hook curved, with separate channels and pores (Fig. 3); abaxial prong channel opening on distal surface of hook, axial prong channel opening on proximal surface of hook. Lateral hook measurements (Fig. 4): A, 91–102 (97 ± 3.9; 3; 8); B, 89–100 (97 ± 3.7; 3; 8); C, 61–78 (70 ± 6.0; 3; 8); D, 139–155 (148 ± 5.3; 3; 8). Medial hook measurements (Fig. 4): A′, 74–96 (86 ± 7.6; 3; 8); B′, 91–100 (95 ± 3; 8); C′, 55–72 (68 ± 6.0; 3; 8); D′, 130–154 (146 ± 7.4; 3; 8). Bases of hooks embedded in musculature of scolex. Cephalic peduncle 309–600 (454 ± 205.9; 2) long. Scolex (including bothridia and cephalic peduncle) 1,017–1,380 (1,198; 2) long by 659–754 (725 ± 38.4; 5) wide. Microthrix pattern not observed.

Immature segments 49 in number, wider than long, becoming longer than wide with maturity; mature segments 2 in number, 1,519–1,866 (1,693 ± 245.5; 1; 2) long by 378–382 (380 ± 3.0; 1; 2) wide, length-to-width ratio 2.1–4.9:1 (3.3 ± 1.4; 1; 2); gravid segments not seen. Testes 90–111 (101 ± 9.3; 2; 4) in number, ap-



Figures 1–5. Line drawings of *Pedibothrium toliarensis* n. sp. 1. Holotype (DAB). 2. Scolex of paratype (USNPC No. 87580). 3. Hooks of paratype (USNPC No. 87580). 4. Illustration of hook measurements taken. 5. Mature segment of paratype (USNPC No. 87580). Note that segment is broken at level of dashed line. 6. Detail of terminal genitalia of segment illustrated in Figure 5.

proximately round, 30–59 (46 ± 7.8; 2; 12) long by 35–54 (48 ± 5.1; 2; 12) wide, filling anterior half of segment and extending to anterior margin of ovary on poral side of segment, absent from postporal region of segment, arranged in 1 plane, 5–6 irregular columns anterior to cirrus pouch (Fig. 5), 2 irregular columns posterior to cirrus pouch on aporal side of segment; vas deferens coiled, median, in middle of segment, entering base of cirrus sac from anterior. Cirrus sac elongated, J-shaped, 373–477 (425 ± 73.6; 2; 2) long by 113 (n = 1) wide; containing coiled cirrus covered with spiniform microtriches; proximal half of cirrus sac submedian and more or less longitudinal, distal half posterior to proximal half, extending laterally towards genital pore. Ovary at posterior end of segment, 339–369 (354 ± 21.5; 1; 2) long by 204–217 (211 ± 9.3; 1; 2) wide, H-shaped in dorsoventral view, cross sections not prepared, weakly follicular, with thin ovarian isthmus; aporal lobe extending anteriorly approximately halfway to level of cirrus pouch; poral lobe extending slightly further anteriorly. Vagina slightly expanded at base, median, extending just anterior to middle of cirrus pouch, then laterally along anterior margin of cirrus pouch to genital pore. Uterus median, thick walled, extending anteriorly from ovarian isthmus to middle of cirrus pouch. Vitellaria follicular; follicles arranged in 2 lateral bands, extending from near anterior of segment to near posterior of segment, interrupted by cirrus sac, not interrupted by ovary; each band consisting of 1 dorsal and 1 ventral column of follicles. Excretory ducts not seen.

The scolex examined with scanning electron microscopy was found to be in poor condition, perhaps having suffered from preservation within the spiral intestine of its host. No useful microthrix data could be obtained from this specimen.

Taxonomic summary

TYPE HOST: Pseudoginglymostoma brevicaudatum (Günther, 1866), short-tail nurse shark.

TYPE LOCALITY: Mozambique Channel off Toliara, Madagascar.

SITE OF INFECTION: Spiral intestine.

SPECIMENS DEPOSITED: Holotype and 1 paratype (DAB); 1 paratype, MNHN No. 563HF, slide 63 CIX; 3 paratypes (1 specimen with scolex, 1 strobilar fragment, and 1 free segment, USNPC No. 87580).

ETYMOLOGY: This species is named for its type locality, Toliara, Madagascar.

Remarks

Pedibothrium toliarensis is a typical member of the genus *Pedibothrium* in that it possesses 4 uniloculated bothridia, bipronged hooks with separate channels in each of the 2 prongs and a J-shaped cirrus sac that is crossed by the vagina. In addition, like all described species in the genus except *P. veravalensis,* it lacks testes on the poral side of the segment between the cirrus sac and the ovary. *Pedibothrium toliarensis* most closely resembles *P. longispine* but can be distinguished from this species on the basis of its greater number of testes (90–111 vs. 54–70), its longer scolex (730–815 vs. 350), and its possession of a double column of testes rather than a single column of testes on the aporal side of the segment between the cirrus sac and the ovary. *Pedibothrium toliarensis* is easily distinguished from *P. servattorum, P. brevispine,* and *P. kerkhami,* in that the axial prongs of its medial and lateral hooks are curved rather than straight. It is further distinguished from *P. servattorum* and *P. brevispine* in that its testes extend posteriorly to the anterior margin of the ovary on the aporal side of the segment, rather than stopping just posterior to the cirrus pouch on the aporal side of the segment. It differs further from *P. kerkhami,* as emended by Caira (1992), in that it has a greater number of testes (90–111 vs. 75). *Pedibothrium toliarensis* differs from *P. globicephalum* and *P. manteri* in that the bases of its medial and lateral hooks touch one another and are shorter than the prongs, rather than being widely separated from one another and much longer than the prongs, and the lateral margins of the loculi do not, in fact perhaps cannot, fold over the distal surfaces of the hooks. Unlike *P. veravalensis, P. toliarensis* lacks testes on the poral side of the segment between the cirrus pouch and ovary; in addition, *P. toliarensis* has a greater number of testes than *P. veravalensis* (90–111 vs. 20–44). The genital pore of *P. toliarensis* is in the posterior half of the segment rather than the anterior half of the segment as in *P. maccallumi,* and *P. toliarensis* has fewer segments than *P. maccallumi* (23–51 vs. 86). Unlike the poorly known *P. lintoni,* the genital pores of *P. toliarensis* alternate irregularly, rather than being unilateral.

Discussion

Toliara, Madagascar, is a new locality record for the shark *P. brevicaudatum.* The specimen from which the tapeworm species described here was collected remains intact at the Institut Halieutique et des Sciences Marines de l'Université de Toliara, in Toliara, Madagascar, as a voucher for this record. This report expands the known distribution of this shark species (see Compagno, 1984) to include the southwest coast of Madagascar. Given the limited collecting that has been conducted throughout the east coast of Africa, we believe this record suggests that this shark species has a broader geographic distribution than has currently been documented.

The discovery of *Pedibothrium toliarensis* brings the total number of species of *Pedibothrium* to 10. With the exception of *P. kerkhami,* verified records for each of these species suggest that each tapeworm species parasitizes only a single species of shark in the orectilobiform family Rhincodontidae. Unfortunately, host records for *P. kerkhami* remain in conflict. Southwell (1911; p. 222) originally reported this species from "a large specimen" of *Chiloscyllium indicum,* and then subsequently reported it (as *Pedibothrium longispine*) from *Galeocerdo arcticus* and *Rhina ancylostoma* (see Southwell, 1930). Baer and Euzet (1962) found specimens consistent with this species in Southwell's material reported to have been collected from *Galeocerdo tigrinum,* and Caira (1992) listed specimens consistent with Southwell's description of *P. kerkhami* from *Stegostoma fasciatum* collected near Balgal, Queensland, Australia. Caira's (1992; p. 303) indication of "*Nebrius ferrugineus* (Lesson, 1830) (=*Ginglymostoma concolor*)" as the type host for *P. kerkhami* is an error and should be disregarded. Given the host specificity of all of the other species of *Pedibothrium,* we believe it unlikely that this species actually parasitizes 5 host species representing 3 different orders of elasmobranchs. We suspect that either more than 1 species of tapeworm is involved, or there has been some confusion with the identity of the hosts from which the material was obtained. However, location and examination of Southwell's material and additional collections are required before the issue of host specificity in this species can be resolved. Specifically, collection of additional specimens of *P. kerkhami* from *C. indicum* would at least serve to verify this shark species as the type host.

With respect to the issue of multiple species of *Pedibothrium* inhabiting the same host species, as seems to be the case for *G. cirratum* and *S. fasciatum* (see Caira, 1992), although we describe only a single species of *Pedibothrium* from *P. brevicaudatum* here, several of the strobilar fragments found along with *P. toliarensis* suggest that 1 and perhaps even 2 additional species of *Pedibothrium* may also parasitize this host species. This additional material included a segment lacking postporal testes on the poral side of the segment but with an extremely posterior genital pore, as well as several strobilar fragments with segments similar in general morphology to those of *P. toliarensis,* but with up to 142 testes. In general, the limited host material examined here gives us little confidence that we have discovered the entire compliment of *Pedibothrium* species hosted by the short-tail nurse shark. These ideas remain to be verified by the examination of additional specimens of *P. brevicaudatum.*

Three of the 5 species of sharks in the family Rhinodontidae are now known to host species of *Pedibothrium.* Given that these 3 species represent each of the 3 lineages of rhincodontids (see Dingerkus, 1986), it seems likely that the other 2 rhincodontid species will also be found to host this tapeworm genus. However, examination of the tapeworm fauna of the whale shark, *Rhincodon typus,* will be especially interesting given that, unlike the 4 other species in the family, the whale shark is a filter feeder rather than a bottom feeder.

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